

TABLE 1. MAA immunophenotyping of melanoma vaccine

Antisera	MAA defined (kilodaltons)	Presence of MAA (vaccine batch)			Ref.
		1	2	3	
Mouse monoclonal					
225.28S	240 +	+	+	+	23
9.2.27	240 +	+	+	+	24
436.G10	122-130	0	NT <sup>b</sup>	NT	25
Nu4B	26, 29, 95, 116	+	NT	NT	17
376.96	94	0	NT	NT	15
118.1	94-97	+	+	+	27
465.12S	94	0	NT	NT	26
McTBT	69-70	0	NT	NT	
Rabbit polyclonal					
SB29, SB54	240	+	+	+	11
SB29, SB54	150 <sup>a</sup>	+	+	+	11
SB29, SB54	140 <sup>a</sup>	+	+	+	11
SB29, SB54	120	+	+	+	11
SB29, SB54	95	+	+	+	11
SB29, SB54	75	+	+	+	11

<sup>a</sup> Not reactive with SB54.<sup>b</sup> (NT) not tested.

**TABLE 2. Effect of detergent and ultracentrifugation on macromolecules, MAAs, and Dr antigens in material shed by melanoma cells**

Treatment <sup>a</sup>	Presence in shed material after ultracentrifugation					
	<sup>125</sup> I-macromolecules <sup>b</sup>		<sup>125</sup> I-MAAs <sup>c</sup>		<sup>125</sup> I-Dr <sup>c</sup>	
	Change <sup>d</sup>		Change		Change	
	cpm	(%)	cpm	(%)	cpm	(%)
None	10,362		817		428	
Ultracentrifugation	6,325	-40	258	-70	0	-100
NP-40 + ultracentrifugation	8,612	-17	574	-30	0	-100

<sup>a</sup> Material shed by radioiodinated melanoma cells was ultracentrifuged in 0.5-ml aliquots at 100,000 g for 90 min, incubated in a final concentration of 0.5% NP-40 for 2 h prior to ultracentrifugation, or not treated. All were subsequently assayed for radioactivity associated with macromolecules, MAAs defined by antiserum SB29, or Dr antigens. All assays were performed on 0.025-ml aliquots of material in the presence of a final concentration of 0.5% NP-40.

<sup>b</sup> Assayed by precipitation with 10% trichloroacetic acid.

<sup>c</sup> Assayed by protein A-immune precipitation with specific antisera.

<sup>d</sup> From untreated control.

TABLE 3. Surface MAAs expressed by melanomas in various individuals

Expression of MAA in melanoma*																
MAA	Antiserum	HM31	HM34	HM49	HM54	HM60	HM80	G361	SK23	SK27	SK28	SK29	SK37	M14	M20	VAL
240 +	SB29	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+
	SB54	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-
	225.28S	-	-	-	-	+	+	-	+	-	+	+	+	+	+	-
	9.2.27	-	-	-	-	+	+	-	+	±	+	+	+	+	-	-
150	SB29	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-
140	SB29	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-
120	SB29	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-
	SB54	+	+	+	+	-	-	+	-	-	-	+	-	-	+	-
116	Nu4B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
95-97	SB29	+	+	-	+	+	-	+	+	+	-	-	-	-	-	-
	SB54	+	+	-	+	-	-	+	+	-	-	-	-	-	-	-
	118.1	-	-	-	-	+	+	-	+	+	+	-	+	+	+	+
	SB29	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
75	SB54	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-
70	Me3 TBT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

\* Assayed by indirect immunoprecipitation with protein A-sepharose.

TABLE 4. Characteristics of immunized patients

Patient no.	Age	Sex	Previous treatment other than surgery	Site of metastasis	Duration of metastatic disease prior to immunization (months)	No. of immunization	Current status <sup>a</sup>	Length of follow-up <sup>b</sup> (months)
1	31	F	BCG, <sup>c</sup> DTIC	Skin, lung	12	10	P	1/4
2	24	F	None	Lung	2	10	P	3
3	53	M	None	Skin	2	13		8
4	58	F	None	Skin	2	8	P	1
5	48	M	None	Skin	1	12	P	3
6	54	M	None	Skin	2	11	P	3
7	58	M	None	Skin	2	14	P	4
8	68	M	None	Skin, lung	2	10	P	6
9	75	F	DTIC, Actinomycin D	Skin	36	17	S	14
10	46	M	None	Skin	1	18	R	24
15	29	M	None	Skin	4	8	P	2
17	68	M	None	Skin	4	8	P	2
20	38	M	None	Skin, lung	3	13	P	4

<sup>a</sup> (P) progression; (S) stable; (R) regression.

<sup>b</sup> From onset of immunotherapy.

<sup>c</sup> (BCG) bacillus Calmette-Guérin.

TABLE 5. *Immunogenicity of melanoma vaccine*

Patient no.	Immune response to melanoma <sup>a</sup>		
	Humoral <sup>b</sup>	Cellular <sup>c</sup>	Either
1	++	0	5
2	+	10	+
3	+	0	+
4	0	0	0
5	±	5	0
6	0	0	0
7	0	0	+
8	0	0	0
9	0	20	+
10	+	10	+
15	++	0	+
17	0	NT	0
20	NT	25	+
No. (%) positive:	5 (38%)	4 (31%)	8 (62%)

<sup>a</sup> (±) 25–49%, ( + ) 50–100%, and ( + + ) 100%, increase over preimmunization level of melanoma antibodies; (NT) not tested.

<sup>b</sup> By indirect immunoprecipitation of <sup>125</sup>I-melanoma macromolecules.

<sup>c</sup> By skin test to 10 µg vaccine; results represent millimeters of average induration at 24–48 h.

**TABLE 6. Antibodies to fetal calf serum proteins in patients immunized to melanoma vaccine**

Patient no.	Antibodies to preimmunization	<sup>125</sup> I-FCS* (2 months' postimmunization)
Melanoma		
1	18.3	0.7
2	0.0	0.1
3	0.0	0.0
4	0.1	0.1
5	0.1	0.2
6	0.1	<0.1
7	<0.1	0.1
8	<0.1	<0.1
9	0.6	0.8
10	0.0	0.0
15	0.1	<0.1
17	0.3	0.4
20	0.0	0.0
Normal		
2003	0.0	
2004	0.1	
2005	<0.1	
2006	0.0	
2007	0.0	
2008	0.0	
2009	0.0	
2010	0.1	
2011	0.0	
2012	0.0	
2013	0.0	
ANTI-FCS	68.0	

\* Percent of radioactivity associated with <sup>125</sup>I-FCS specifically immunoprecipitated by serum and protein A-sepharose.